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Cytogenetic mapping of common bean chromosomes reveals a less compartmentalized small-genome plant species

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Abstract Cytogenetic maps of common bean chromosomes 3, 4 and 7 were constructed by fluorescence in-situ hybridization (FISH) of BAC and a few other genomic clones. Although all clones were selected with genetically mapped markers, mostly with single-copy RFLPs, a large subset of BACs, from 13 different genomic regions, contained repetitive sequences, as concluded from the regional distribution patterns of multiple FISH signals on chromosomes: pericentromeric, subtelomeric and dispersed. Pericentromeric repeats were present in all 11 chromosome pairs with different intensities, whereas subtelomeric repeats were present in several chromosome ends, but with different signal intensities depending on the BAC, suggesting that the terminal heterochromatin fraction of this species may be composed of different repeats. The correlation of genetic and physical distances along the three studied chromosomes was obtained for 23 clones. This correlation suggests suppression of recombination around extended pericentromeric regions in a similar way to that previously reported for plant species with larger genomes. These results indicate that a relatively small plant genome may also possess a large proportion of repeats interspersed with single-copy sequences in regions other than the pericentromeric heterochromatin and, nevertheless, exhibit lower recombination around the pericentromeric fraction of the genome.

Keywords fluorescence in-situ hybridization (FISH) · bacterial artificial chromosome (BAC) · cytogenetic map · recombination · Fabaceae

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Abbreviations

^{32}P	radioactive isotope of phosphorus
APA loci	family of seed proteins consisting of α-amylase inhibitors (α-AI), phytohaemagglutinins (PHA) and arcelins (ARL)
BAC	bacterial artificial chromosome
Bng	(common) bean genomic plasmid clone
C_0t	C_0 is the initial concentration of single stranded DNA in mol/l; t is the reannealing time in seconds
CCD	charge-coupled device
CIAT	International Center for Tropical Agriculture
cM	centimorgan
Cy3-dUTP	5-aminopropargyl-2′-deoxyuridine 5′-triphosphate coupled to red cyanine fluorescent dye
DAPI	4′,6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
FISH	fluorescence in-situ hybridization
*Hin*dIII	restriction enzyme from *Haemophilus influenzae*
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
SD	standard deviation
SDS	sodium dodecyl sulfate
SSC	saline-sodium citrate buffer (20× SSC: 3 M NaCl, 0.3 M sodium citrate, pH 7.0)
SSPE	saline-sodium phosphate-EDTA buffer (20× SSPE: 3 M sodium chloride, 0.2 M sodium hydrogenphosphate, 0.02 M EDTA, pH 7.4)
TAC	transformation-competent artificial chromosome

Introduction

The common bean (*Phaseolus vulgaris* L.) is the major legume crop for direct consumption, and is an important source of dietary proteins for populations in Africa and Latin America. In an effort to accelerate improvement of the common bean, the Phaseomics consortium has been established, bringing together bean researchers from different fields. One of the aims of this consortium is to develop genomic resources for the species (Broughton et al. 2003). A physical map for the common bean was also considered to be a priority among researchers from the legume community and should be established by 2011 (Gepts et al. 2005). Recently, a draft physical map of *P. vulgaris* cv. G19833 was developed, with 34 264 clones assembled into 1183 contigs and the remaining 6385 clones existing as singletons (Schlueter et al. 2008).

To date, several genetic maps are available for the common bean (Gepts et al. 2008). This is a diploid species with 22 chromosomes and a haploid genome size estimated to be around 600 Mb (Arumuganathan and Earle 1991; Bennett and Leitch 1995). Linkage groups from previously developed genetic maps (Vallejos et al. 1992; Freyre et al. 1998) have been assigned to the corresponding chromosome pairs by pooling RFLP markers from each linkage group and hybridizing them *in situ* to chromosomes (Pedrosa et al. 2003). This study provided a set of markers that allowed the identification of each chromosome of the species. It also revealed a lack of correlation between chromosome and linkage group sizes. However, the differences in recombination frequencies among and along chromosomes could not be investigated because linkage groups and chromosomes were not connected by multiple shared sequences: only one probe, composed of several RFLP markers of the same linkage group, was available for most chromosomes.

Large-insert BAC clones have frequently been used in fluorescence in-situ hybridization (FISH) experiments to physically map single-copy, genomic sequences to chromosomes of plants with a small genome size, such as *Arabidopsis* (Fransz et al. 2000), rice (Cheng et al. 2001a,b), sorghum (Kim et al. 2005a,b), *Medicago truncatula* (Kulikova et al. 2001) and *Lotus japonicus* (Pedrosa et al. 2002). Since several BAC clones have been selected using genetically mapped markers and mapped by FISH along chromosomes, genetic and physical distances could be compared and associated with chromosome landmarks such as centromeres, telomeres and heterochromatin. In the present work, we established cytogenetic maps based on fluorescence in-situ hybridization of BACs and a few other genomic clones for the first three common bean chromosomes. These maps enabled the evaluation of recombination frequencies along these chromosomes, gave an

overview of the common bean genome organization and provided anchoring points for future contig maps.

Materials and methods

Plant material

Seeds from the *P. vulgaris* Mesoamerican breeding line BAT93 were obtained from the germplasm bank of the International Center for Tropical Agriculture - CIAT, Cali, Colombia.

Chromosome preparation

Root tips obtained from germinating seeds were pre-treated in 2 mM 8-hydroxyquinoline for 5 h at 16°C, fixed in ethanol-acetic acid 3:1 (v/v) and stored in fixative at −20°C for up to several weeks. Mitotic chromosome preparation was performed as described in Pedrosa-Harand et al. (2006), with maceration in 3% (w/v) cellulase 'Onozuka R-10' (Serva, Heidelberg, Germany) plus 30% (v/v) pectinase (Sigma-Aldrich, St. Louis, MO, USA) in 0.01 M citric acid-sodium citrate buffer pH 4.8 for 1.5 h at 37°C. In order to confirm the orientation of clones mapped to the short arm of chromosome 7, young flower buds were fixed as described above for pachytene chromosome preparation. Pachytene chromosome spreads were prepared according to Armstrong et al. (2001), except that whole flower buds were digested in 0.33% (w/v) cellulase 'Onozuka R-10' (Serva) and 0.33% (w/v) pectolyase (Sigma-Aldrich) for 3 h at 37°C, and meiocytes were left in 60% acetic acid at 45°C for a few seconds before re-fixation.

DNA probes

BAC clones were selected by screening high-density BAC filters from a BAT93 *Hin*dIII genomic library (Kami et al. 2006) using genetically mapped markers. BACs corresponding to the *Phaseolin* and APA loci were selected by Kami et al. (2006). BACs corresponding to RFLP clones (*Bng*s) mapped to linkage groups A, B and C (Vallejos et al. 1992) were selected using PCR fragments amplified as described in Pedrosa et al. (2003). The PCR products were purified by ethanol precipitation and labelled with ^{32}P using the random primer method. The labelled probe was purified in a Sephadex G-50 column (Sambrook et al. 1989). Filters were hybridized for 16 h at 65°C in 7.5% SDS, 5× SSPE and washed with 2× SSC-0.1% SDS, 1× SSC-0.1% SDS, 0.5× SSC-0.1% SDS and 0.1× SSC-0.1% SDS at 65°C. Radioactivity signals were detected using a FX Molecular Imager (BioRad, Hercules, CA, USA). Each probe was individually hybridized to one filter and was used for a second hybridization only if no positive clone was identified in the first trial. Bacteriophages co-localized at the end of linkage group B4 with a complex disease resistance gene cluster (Geffroy et al. 2008, 2009) were selected as described by Ferrier-Cana et al. (2003). BAC and bacteriophage DNA were isolated using the Plasmid Mini Kit (Qiagen, Hilden, Germany) and Nucleobond AX columns (Macherey-Nagel, Düren, Germany), respectively, following the manufacturer's instructions. All selected genomic clones were labelled by nick translation (Roche Diagnostics, Indianapolis, IN, USA) with Cy3-dUTP (GE Healthcare, Bucks, UK) or SpectrumGreen-dUTP (Vysis, Des Plaines, IL, USA).

DNA dot blot analysis

DNA (10 ng) from BAC clones that showed strong positive signals after library screening was spotted onto an N+ Hybond membrane. Hybridization of the C_0t-1 fraction, isolated according to Zwick et al. (1997), was performed with the ECF random priming labelling and signal amplification system (GE Healthcare) according to the manufacturer's instructions. Fluorescent signals were detected using an FX Molecular Imager with an external laser of 488 nm and ECF settings (BioRad).

Fluorescence In-Situ Hybridization (FISH)

Slides were selected and pre-treated as described in Pedrosa et al. (2001). Chromosome and probe denaturation, post-hybridization washes and detection were performed according to Heslop-Harrison et al. (1991), except for the stringent wash, which was performed with 0.1× SSC at 42°C. Hybridization mixes consisted of: 50% (v/v) formamide, 10% (w/v) dextran sulfate, 2× SSC and 2–5 ng/μl probe. *P. vulgaris* C_0t-1 or C_0t-100 fractions were added in 5 to 100-fold excess to the hybridization mix to block repetitive sequences where necessary. The

mitotic and meiotic preparations were denatured for 5 min at 75°C and 3 min at 73°C, respectively. All slides were hybridized for up to three days at 37°C. Preparations were counterstained and mounted with 2 μg/ml DAPI in Vectashield (Vector, Burlingame, CA, USA). Re-probing of slides for localization of different DNA sequences on the same cell was performed according to Heslop-Harrison et al. (1992), up to five times.

Data analysis

Photographs were taken on a Zeiss Axioplan (Carl Zeiss) microscope equipped with a mono cool view CCD camera (Photometrics, Tucson, AZ, USA) and the IPLab spectrum software (IPLab, Fairfax, VA, USA). The ten best mitotic metaphases bearing clear hybridization signals were selected in order to calculate the position of each clone by measuring the distance of the centre of the signal(s) to the closest telomere and the total chromosome length in arbitrary units. Relative clone position was represented by the mean value of all measurements, with 0 representing the telomere of the short arm and 1 the telomere of the long arm. Relative chromosome size and arm ratio were calculated based on measurements of at least five mitotic metaphases. All measurements were performed using the 'analyse - measure length' function of the IPLab software. Assignment of a clone to a specific chromosome arm was confirmed by re-probing the slides with a previously mapped clone. Chromosomes were named and oriented according to the standard common bean nomenclature (Freyre et al. 1998; Pedrosa-Harand et al. 2008). Images were superimposed using the same software and artificial colours and imported into Adobe Photoshop version 8 for final processing.

Results

DNA markers from the Florida linkage map of the common bean (Vallejos et al. 1992) were chosen for screening the 'BAT93' BAC library (Kami et al. 2006) because its RFLP markers (*Bng* clones) are widely available and this was one of the three maps integrated into the core map of the species (Freyre et al. 1998). A total of 16, 8 and 17 markers from linkage groups A, B and C, respectively, were used for the BAC library screening. These groups correspond to linkage groups B7, B4 and B3, respectively, of the core map (Freyre et al. 1998). The aim was to select at least 10 BAC clones for each linkage group, except for linkage group B, which had only 8 RFLP markers mapped. However, BACs corresponding to only 8, 6 and 9 markers, respectively, were identified, suggesting a lower coverage of the library for some genomic regions. BAC clones for two additional genomic regions, the *Phaseolin* locus from linkage group A and the APA locus from linkage group B, were previously selected (Kami et al. 2006) and also localized by FISH (Table 1).

A high proportion of the selected BACs with single-copy markers (BACs for 13 out of 25 genomic regions) did not show unique, localized signals in just one chromosome pair, as expected. Instead, three general patterns of labelling the chromosome complement were observed, suggesting the presence of repetitive sequences within their inserts. Indeed, dot blot hybridization of BAC DNA on a membrane and C_0t-1 repetitive fraction of *P. vulgaris* DNA as a probe showed stronger hybridization for those BACs than for BACs that showed unique signals after FISH (Table 1). The most common, distinct in-situ hybridization patterns were designated as subtelomeric (labelling of chromosome ends) and pericentromeric (labelling around centromeric regions) (Fig. 1a, b). In a few FISH experiments, a C-banding-like staining of the chromosomes was observed with DAPI. The detected terminal blocks corresponded to the strongest subtelomeric signals (see Supplementary Fig. S1a–d). Positive bands were also observed in centromeric regions of most chromosomes, but these bands were smaller than the pericentromeric signals observed with pericentromeric BAC clones (Supplementary Fig. S1a, e–f). This suggests that the pericentromeric BACs label beyond the pericentromeric heterochromatin. A similar hybridization pattern was observed for all BACs showing a pericentromeric pattern, although they have been selected with five different mapped markers. All BACs labelled all chromosomes of the complement, but chromosomes showed different labelling intensities. The three chromosome pairs showing the weakest hybridization signals were submetacentric and acrocentric in morphology. For the four subtelomeric BACs, three subtypes of hybridization pattern were observed. They labelled a subset of chromosome arms only and the intensity of

Table 1 List of mapped markers and genes used for screening BAC clones and the general pattern of hybridization of the selected BACs after dot blot and FISH

Linkage group	Marker/gene	BAC clone	Dot blot[a,b]	FISH pattern without blocking DNA[a]	FISH pattern with blocking DNA[a]
A	*Bng 23*	20F21	NA	NA	NA
		33M20	+++	Unique + weakly scattered	Unique (5×C_0t-1)
	Bng 28	12M3	+++	Pericentromeric	NA
		22I21	+	Unique	Unique (50×C_0t-100)
	Bng 42	193F10	−	Unique	Unique (5×C_0t-1)
		200B23	++	NA	NA
		202F18	+	NA	NA
		215P4	−	NA	NA
	Bng 47	267K20	NA	Unique	NA
	Bng 60	144D16	+	Unique	Unique (5×C_0t-1)
	Bng 170	200E15	+++	Pericentromeric	Pericentromeric (50×C_0t-100)
	Bng 191	86I17	+++	Subterminal	Subterminal (50×C_0t-100)
	Bng 204	111O19	NA	Disperse	NA
		122D11	+++	NA	Unique (100×C_0t-1)
		125P11	NA	Disperse	NA
	Phs	101J20	+++	NA	NA
		105O5	+++	Pericentromeric	Pericentromeric (75×C_0t-1)
B	*Bng 13*	25C4	NA	NA	NA
		26A21	NA	NA	NA
		26B20	+	Unique + weakly scattered	Unique (20×C_0t-1)
		30E20	+	NA	NA
		31N7	++	NA	NA
		35K5	NA	NA	NA
		38C19	NA	NA	NA
		47A5	NA	NA	NA
	Bng 55	53G1	+++	Pericentromeric	Pericentromeric (50×C_0t-100)
		77B3	+++	Pericentromeric	Pericentromeric (50×C_0t-1)
		92B6	NA	NA	NA
		92P15	NA	NA	NA
	Bng 103	162K15	+	Unique + weakly scattered	Unique (20×C_0t-1)
	Bng 151	221J10	+++	Subterminal	Unique (50×C_0t-100)
	Bng 160	53N15	NA	NA	NA
		75K8	NA	NA	NA
		75H11	NA	NA	NA
		86B17	NA	NA	NA
		76N21	+++	NA	NA
		78L24	+++	Subterminal	Subterminal (50×C_0t-100)
		93F6	+++	NA	NA
		94F8	NA	NA	NA
	Bng 184	165P21	NA	NA	NA
		187E12	+++	NA	NA
		190C15	++	Subterminal	Unique (50×C_0t-1)
	APA	86K9	+	Unique	NA

Table 1 (continued)

Linkage group	Marker/gene	BAC clone	Dot blot[a,b]	FISH pattern without blocking DNA[a]	FISH pattern with blocking DNA[a]
		214L14	+++	NA	NA
C	*Bng 3*	60H5	NA	NA	NA
		72D12	NA	NA	NA
		77J14	+	Disperse	Unique (50×C_0t-100)
		92D1	+	NA	NA
	Bng 12	142D9	+	Unique	NA
	Bng 16	253F1	++	NA	NA
		267H4	+	Disperse	Unique (50×C_0t-100)
		284P11	NA	NA	NA
		285J8	NA	NA	NA
	Bng 33	95L13	+	Unique	NA
		174E13	+	Unique	NA
	Bng 63	287I5	+++	Pericentromeric	NA
	Bng 75	199D13	+	Unique + weakly scattered	Unique (50×C_0t-100)
		220C15	+	NA	NA
		230M3	NA	NA	NA
		234H24	NA	NA	NA
	Bng 106	147K17	+	Unique	NA
		165K4	NA	NA	NA
		173L21	+	NA	NA
		180D23	NA	NA	NA
	Bng 114	116H6	++	Disperse	Unique (50×C_0t-100)
	Bng 124	91K16	+	Unique	NA

[a] NA, not analysed.

[b] Intensity of signals: +++, strong; ++, medium; +, weak; -, very weak or not detected.

the labelling at different chromosome ends varied depending on the BAC clone used (Supplementary Fig. S1b–d). Five BACs, from four different genomic regions, showed a more dispersed labelling of the complement, sometimes showing stronger signals in proximal chromosome regions (closer to centromeres than to telomeres). Those BACs, as well as two subtelomeric ones (BACs 190C15 and 221J10), could be mapped after addition of blocking DNA in the hybridization mix. Blocking DNA was also added when a unique signal could be discerned but a weaker, scattered labelling of the complement was also observed (Table 1).

A total of 23 clones (19 BACs, three bacteriophages and one plasmid clone) showed unique and localized signals on chromosomes with or without the addition of blocking DNA. These clones were mapped on mitotic metaphase chromosomes because at this stage chromosomes reach the maximum degree of condensation and it is expected that both euchromatin and heterochromatin are similarly condensed, allowing a more precise positioning of clones along the chromosome length. Pachytene chromosomes offer a higher mapping resolution, but positioning of clones along the chromosome is influenced by its heterochromatin content and degree of condensation. Furthermore, as was observed in the common bean, the chromosome pairs observed at pachytene stage may be entangled, hampering the measurement of each chromosome from one end to the other and, thus, the estimation of the relative position of each clone. The 23 clones were mapped to three chromosomes pairs, which were named 3, 4 and 7 according to the nomenclature of the corresponding linkage groups proposed by Freyre et al. (1998) and defined

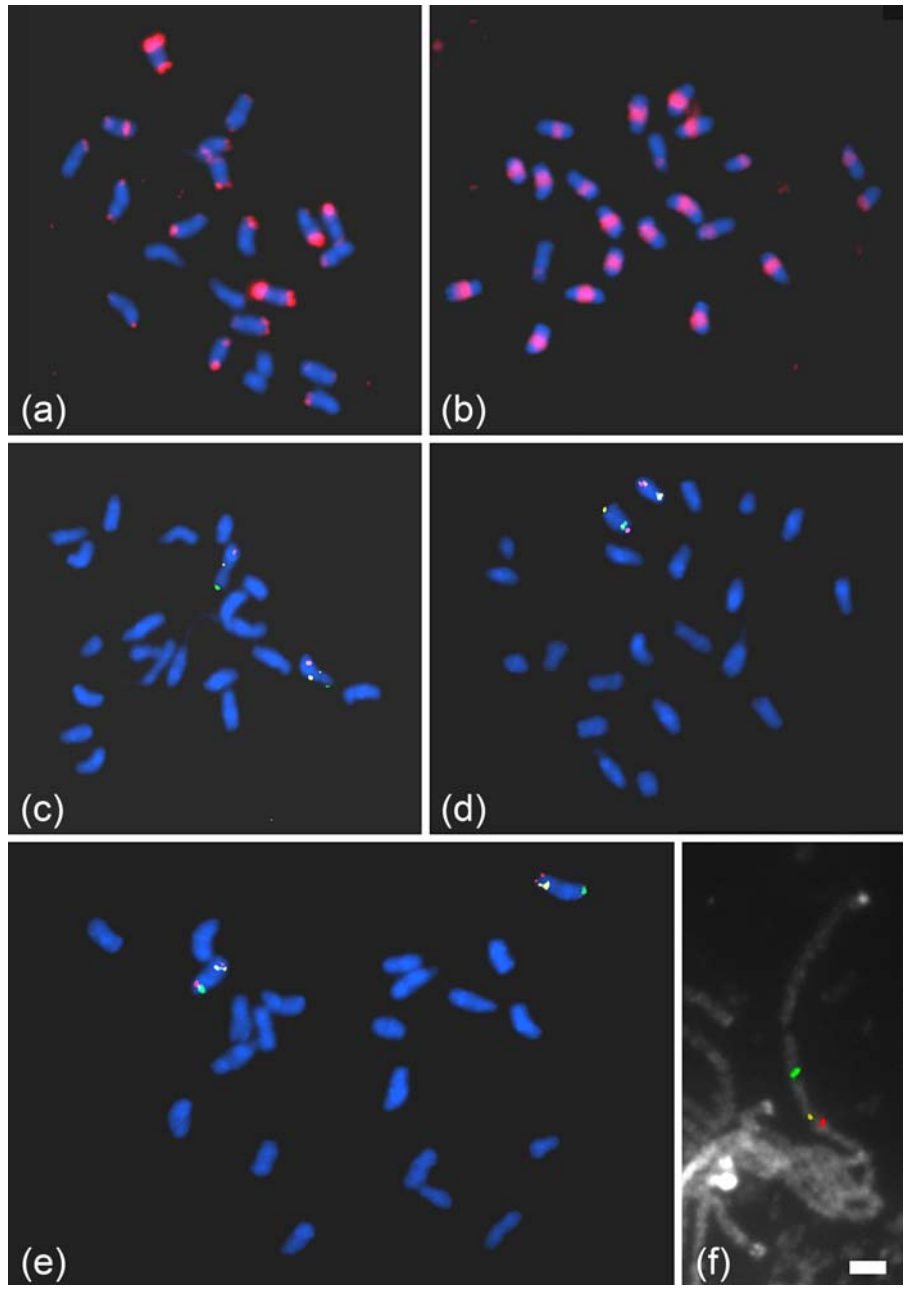

Fig. 1 In-situ hybridization of genetically assigned clones on *P. vulgaris* BAT93 mitotic **(a–e)** and pachytene **(f)** chromosomes. **(a)** 78L24 (red). **(b)** 77B3 (red). **(c)** BACs mapped on chromosome 3: 147K17 (red), 267H4 (pink), 199D13 (yellow) and 91K16 (green). **(d)** Clones mapped on chromosome 4: B35 (yellow), 26B20 (red), 162K15 (green) and 86K9 (pink). **(e)** Clones mapped on chromosome 7: 193F10 (green), 144D16, *Phaseolin* and 267K20 (simultaneously in red) and 33M20 (yellow). **(f)** Higher resolution mapping of 193F10 (green), 144D16 (yellow) and *Phaseolin* (red). All chromosomes were counterstained with DAPI and visualized in blue, except in (f) were they are seen in grey. Bar in (f) represents 2.5 μm

as the standard nomenclature for common bean chromosomes (Pedrosa-Harand et al. 2008). Chromosomes 3, 4 and 7 were metacentric, with arm ratios of 1.85, 1.47 and 1.69, respectively. Chromosome 7 was the largest, with chromosomes 3 and 4 having relative lengths of 0.97 and 0.86 compared to chromosome 7.

BACs corresponding to eight mapped markers were included in the cytogenetic map of chromosome 3 (Figs. 1c, 2 and 3, and Table 2). Four of these BACs were mapped with the help of C_0t-1 or C_0t-100 blocking DNA from *P. vulgaris*. Three BACs mapped to the short arm and five to the long arm of this chromosome. The centromere was located between BACs 267H4 (*Bng*16) and 199D13 (*Bng*75). The genetic distance between these two markers was 6.3 cM (6.66% of the total linkage group length of 94.6 cM), but the physical distance between the respective BAC clones corresponded to one-third of

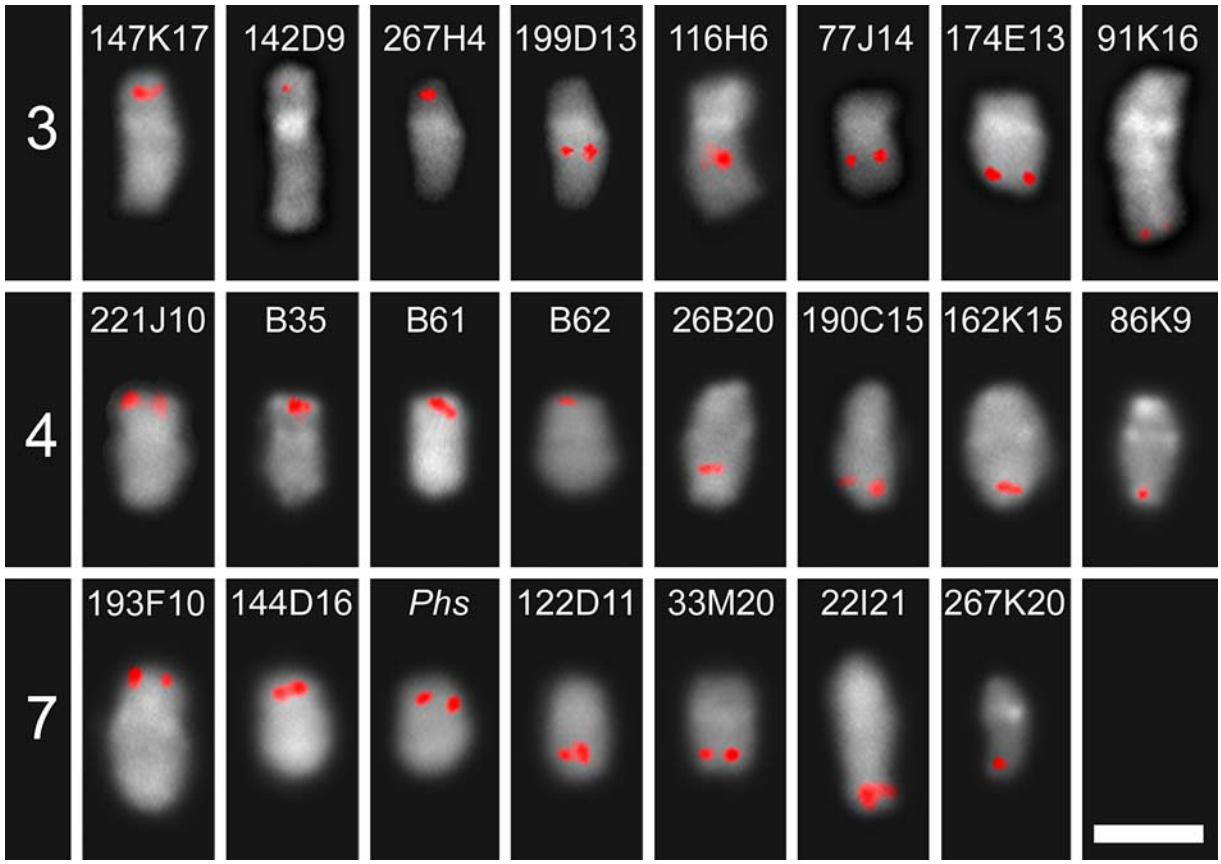

Fig. 2 Localization of clones (red signals) on *P. vulgaris* BAT93 mitotic chromosomes (grey). One chromosome is shown for each mapped clone. Clones are ordered according to the chromosomes they belong to and to their position in the cytogenetic map. Bar represents 2.5 μm

the total chromosome length, clearly indicating suppression of recombination around this centromere.

Five BACs were mapped on chromosome 4 (Figs. 1d, 2 and 3, and Table 2). These BACs were located on the distal half of the short and long chromosome arms. Three bacteriophage clones (B35, B61 and B62), co-localized with a disease resistance gene cluster at the end of linkage group B4, were also mapped to the short arm of chromosome 4 (Geffroy et al. 2009). A BAC (77B3) selected with an interstitially mapped marker revealed a highly repetitive, pericentromeric distribution (Fig. 1b) that could not be blocked, although both C_0t-1 and C_0t-100 DNA were tested, as well as another BAC (53G1) from the same genomic region (data not shown). Similarly, a BAC (78L24) corresponding to one of the most distal markers of the short arm (*Bng*160) could not be mapped. This BAC labelled the subtelomeric region of most chromosome arms (Fig. 1a), with the strongest signals on the short and long arms of chromosome 4. Two other subtelomeric BACs from this chromosome (190C15 and 221J10) also labelled the subtelomeric region of more than one chromosome pair, but the number of subtelomeres labelled, as well as the relative intensity of signals among chromosome ends, were different among these three BACs. These differences in hybridization pattern suggest the presence of different subtelomeric repeats in the common bean genome.

The map of chromosome 7 includes six BACs and a clustered, low-copy-number, gene family (*Phaseolin*), which was mapped with a plasmid that contains one copy of this gene. The *Phaseolin* clone and two BACs were placed on the short arm and four BACs were mapped to the long arm (Fig. 1e, 2 and 3, and Table 2). Higher-resolution mapping of the short arm of chromosome 7 was performed on pachytene chromosomes (Fig. 1f) and confirmed the position of

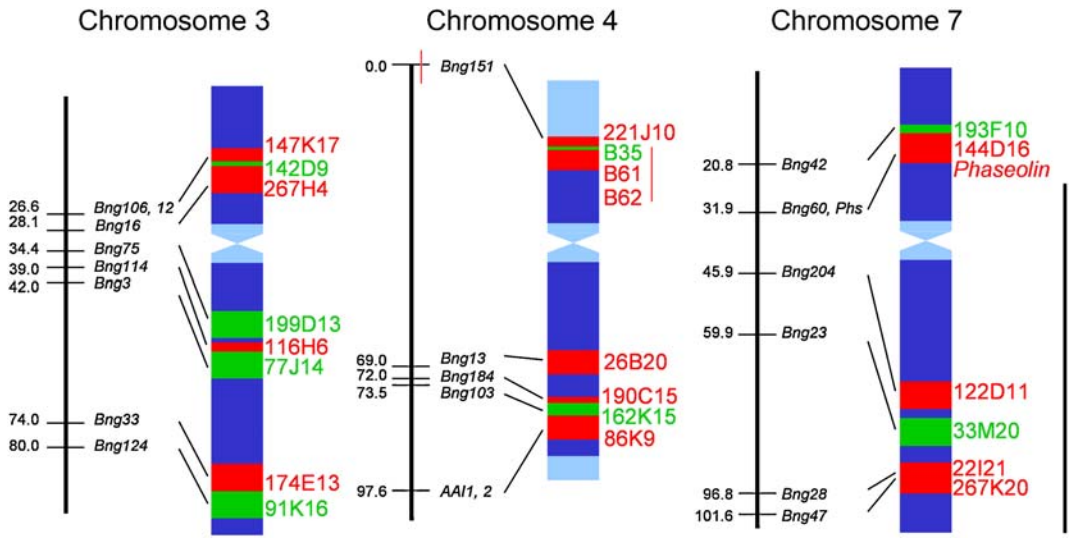

Fig. 3 Diagrammatic representation of chromosome 3, 4 and 7 cytogenetic maps (right) and genetic position of the corresponding molecular markers (left). Clones are indicated in red and green, and chromosomes in blue. Light blue blocks represent approximate location of constitutive heterochromatin as visualized as brighter-stained regions on mitotic chromosomes after FISH experiments. Chromosomes and linkage groups are drawn to scale, respectively. Positions of genetic markers in cM are derived from the map of Vallejos et al. (1992). All three linkage groups were rotated top to bottom with respect to the original map to better represent the correspondence of markers to the short and long chromosome arms. Bar on the right represents 2.5 μm

the corresponding clones measured from mitotic chromosomes (Table 2). Again, a suppression of recombination around the pericentromeric region was observed: half of the chromosome (around the centromere) is represented by less than 15% of the linkage group length.

Discussion

In the present work, we have cytogenetically mapped common bean chromosomes 3, 4 and 7 using BACs and other genomic clones through FISH. The established maps reveal important features of the common bean chromosomes, such as suppression of recombination around extended pericentromeric regions and the frequent presence of repetitive sequences interspersed with single-copy sequences in pericentromeric, interstitial and subtelomeric regions.

All BAC clones used in the present work were selected with single-copy, genetically mapped markers. Nevertheless, selected BACs for more than half of the markers used showed a repetitive pattern of hybridization after FISH, indicating the presence of interspersed repeats associated with single-copy sequences within the BAC insert. Dot blot hybridization was an efficient strategy for selecting BACs containing lower amount of repetitive DNA, especially when more than one BAC clone was available for the same, or closely mapped, genetic markers. Southern blot hybridization has been used for the same purpose (Kim et al. 2005b), but requires BAC DNA digestion, electrophoresis and blotting. Other alternatives may be selection of BACs with high gene content (Kim et al. 2005a), selection of BACs corresponding to markers from regions of high genetic recombination (Wang et al. 2007), and subcloning of BACs followed by selection of subclones devoid of major repetitive DNA sequences (Pedrosa et al. 2002; Janda et al. 2006).

When no other BAC was available for the same genetic position, BACs containing repetitive sequences were used as a probe and blocking DNA was added to the hybridization mix in different proportions to suppress the hybridization of the repetitive sequences, leaving unique signals from the single-copy part of the insert. Only 6 of the 11 tested BACs could be successfully mapped after addition of blocking DNA, mainly those originally showing a less intense, more dispersed signal. None of the BACs showing a pericentromeric distribution could be cytologically mapped, possibly because of the high

Table 2 Genetic locations of markers and physical locations of their associated BACs on the respective linkage groups and mitotic metaphase chromosomes

Genetic map		Physical map			
Marker	Position[a]	Clone	Mean	*n*	SD[b]
Chromosome 3/C					
Bng 106	0.28	BAC 147K17	0.17	28	0.04
Bng 12	0.28	BAC 142D9	0.20	27	0.04
Bng 16	0.30	BAC 267H4	0.21	20	0.05
Bng 75	0.36	BAC 199D13	0.53	28	0.05
Bng 114	0.41	BAC 116H6	0.60	24	0.04
Bng 3	0.44	BAC 77J14	0.62	24	0.04
Bng 33	0.78	BAC 174E13	0.87	29	0.06
Bng 124	0.85	BAC 91K16	0.93	23	0.04
Chromosome 4/B					
Bng 151	0.00	BAC 221J10	0.13	27	0.04
–	–	B35[c]	0.14	33	0.05
–	–	B61[c]	0.18	32	0.07
–	–	B62[c]	0.19	34	0.07
Bng 13	0.66	BAC 26B20	0.70	21	0.06
Bng 184	0.69	BAC 190C15	0.82	26	0.05
Bng 103	0.71	BAC 162K15	0.83	26	0.06
APA	0.94	BAC 86K9	0.87	22	0.07
Chromosome 7/A					
Bng 42	0.20	BAC 193F10	0.15	32	0.06
Bng 60	0.30	BAC 144D16	0.17	39	0.05
Phaseolin	0.30	Phs	0.18	34	0.06
Bng 204	0.44	BAC 122D11	0.70	26	0.08
Bng 23	0.57	BAC 33M20	0.78	36	0.05
Bng 28	0.93	BAC 22I21	0.88	28	0.05
Bng 47	0.97	BAC 267K20	0.88	26	0.06

[a] Position of a genetic marker in the genetic map is indicated as percentage of total linkage group length, calculated from data presented by Vallejos et al. (1992). Position in cM is indicated in Fig. 3.

[b] Standard deviation.

[c] See Geffroy et al. 2009.

proportion of repetitive sequences within the BAC insert or because of the too high amount of these repetitive sequences in the genome. Heterochromatic regions are indeed difficult to map by FISH due to an excess of repetitive sequences present in BACs from these regions (Kim et al. 2005b). Addition of C_0t-1 DNA may help to get single copy signals, but it is not effective in all cases (Wang et al. 2007). In potato, even 200-fold excess of blocking DNA was not enough for enabling the mapping of a few BACs tested (Dong et al. 2000).

Although those BAC clones containing a higher proportion of repetitive sequences could not be mapped, they were useful in contributing to the genomic characterization of the common bean. The high proportion of BACs selected with single copy sequences that showed a repetitive hybridization pattern suggested the presence of a high proportion of interspersed repetitive sequences in the common bean genome. Analysis of 89 017 BAC-end sequences and 1404 shotgun sequences estimated that 49.2% of the common bean genome is composed of repetitive sequences, a higher proportion than estimated for other legume genomes, such as *Trifolium repens* or soybean (Schlueter et al. 2008). Furthermore, these results also indicated that repetitive sequences are not as compartmentalized within the common bean genome as in other plant species, such as *Arabidopsis thaliana*, *Medicago truncatula*, *Sorghum bicolor* and *Solanum lycopersicum* (Fransz et al. 1998; Kulikova et al. 2001; Kim et al. 2005a; Wang et al. 2006). In those species, repetitive sequences are present at pericentromeric heterochromatin, but euchromatic regions, encompassing the chromosome arms, are greatly devoid of repeats. In rice, although heterochromatin is mostly located in pericentromeric regions, terminal heterochromatic knobs were detected in the *indica* variety Zhongxian 3037 (Cheng et al. 2001a). Nevertheless, all 18 RFLP-selected BACs for chromosome 10 of rice could be mapped by FISH (Cheng et al. 2001b). The 600 Mbp common bean genome (Arumuganathan and Earle 1991; Bennett and Leitch 1995) is only around 4 times larger than the *Arabidopsis* genome (125 Mbp; TAGI 2000) and 50% larger than the rice genome (389 Mbp; IRGSP 2005). Nevertheless, it is smaller than the genomes of sorghum (818 Mbp; Price et al. 2005) and tomato (950 Mbp; Arumuganathan and Earle 1991), indicating that genome size alone cannot always successfully predict genome structure in plants. Random and TAC-end sequencing in another small-genome legume, *Lotus japonicus* (472 Mbp), also suggested that the gene spaces in this species are intermingled with repeated sequences (Sato et al. 2008).

The distribution patterns observed with pericentromeric and subtelomeric BACs in the present work can

improve the heterochromatin characterization available so far for the species (Zheng et al. 1991; Moscone et al. 1999). These previous studies established the heterochromatin distribution in the common bean based on C-banding. Heterochromatin amount was estimated at 10% of the total chromosome length and heterochromatic bands were observed in centromeric and a few terminal regions (Moscone et al. 1999). Reproducibility and resolution were low, however, and it was not possible to assign heterochromatic bands to specific chromosomes. BACs that labelled pericentromeric regions, such as BAC 77B3, hybridized to pericentromeric regions at very different intensities, in agreement with the presence of heterochromatic blocks of different sizes at different chromosomes of the species. On the other hand, the presence of a subtelomeric heterochromatin in the common bean was more evident after the detection of subtelomeric signals in most chromosome ends with BACs such as 78L24, than with previous C-banding experiments. This subtelomeric heterochromatin corresponds to terminal knobs seen on pachytene chromosomes (see Fig. 1f). Furthermore, three slightly distinct subtelomeric hybridization patterns were detected with BACs 78L24, 190C15 and 221J10, suggesting the existence of at least three types of repetitive sequences composing the subtelomeric heterochromatin of the common bean. Subcloning and sequencing will be necessary to further characterize this genome fraction, as performed in wheat (Zhang et al. 2004).

High suppression of recombination, comprising at least one-third of the proximal chromosomal regions, was observed in the common bean, especially for chromosomes 3 and 7, for which a larger number of BACs was available. In species with small genomes, such as *Arabidopsis* and rice, a relatively uniform distribution of recombination events has been observed, with suppression of recombination restricted to a few Mbp around the centromeres, where most of their repetitive DNA is present (Schmidt et al. 1995; Cheng et al. 2001b). On the other hand, strong suppression of recombination comprising large proximal regions has been demonstrated in wheat (Gill et al. 1996) and barley (Künzel et al. 2000), species with large genomes (approximately 16 000 Mbp (2n=6x) and 5000 Mbp, respectively (Arumuganathan and Earle 1991)). In potato (approximately 1700 Mbp (2n=4x) (Arumuganathan and Earle 1991)) and tomato, suppression of recombination was associated with the prominent pericentromeric heterochromatin (Sherman and Stack 1995; Iovene et al. 2008; Tang et al. 2008). In sorghum, a species with a genome approximately twice as large as the rice genome, heterochromatin comprises around 50% of its genome and is restricted to proximal chromosomal regions. Again, recombination was largely suppressed, approximately 34-fold lower, in those regions compared with euchromatic regions (Kim et al. 2005a). Although the precise heterochromatin-euchromatin border in the common bean has not been established, the suppression of recombination in extended pericentromeric regions was evident in this species, indicating that this phenomenon can also be observed in a plant with a relatively small and less compartmentalized genome. The cytogenetic maps of the remaining chromosomes, currently under construction, will confirm whether the suppression of recombination is present in all common bean chromosomes.

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